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Production of a Novel OX40 Ligand for Clinical Use

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14. ABSTRACT
Cancer cells have evolved to evade immune-mediated destruction through several documented mechanisms. Our group has developed a technique to enhance immune function in tumor-bearing hosts by targeting a protein on the surface of white blood cells, termed OX40. This type of immune modulation leads to therapeutic benefit in tumor-bearing mice. We have produced a protein that binds to the human OX40 protein and activates human white blood cells. We have a cell line that produces high quantities of this protein and our goal is to test this protein for safety and efficacy in non-human primates so that we can obtain FDA approval for clinical trials in cancer patients. The long-range goal of this proposal is translate these findings to prostate cancer patients.

15. SUBJECT TERMS
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DOD Prostate Award Research Technical Reporting: 2 year Progress Report

Laboratory-Clinical Transition Award:

PC101977 – Production and Characterization of a Novel OX40 Ligand for Clinical Use.

PI -Andrew Weinberg, PhD

INTRODUCTION: Cancer cells have evolved to evade immune-mediated destruction through several documented mechanisms. Our group has developed a technique to enhance immune function in tumor-bearing hosts through the use of OX40 agonists, which can lead to regression of tumors of various histologies, including prostate cancer. In particular, we have produced a human OX40 agonist, termed OX40L:ILZ:Ig (OX40L:Ig) that has potent biologic function in vitro and is produced in large quantities by tissue culture cells. The ILZ portion of the chimeric protein was initially a trimerization domain obtained from a yeast sequence. In the past few years we have produce a fully human OX40 ligand protein and it was tested for in vivo biologic activity in non-human primates and had potent activity. The ultimate goal of the current research is to produce clinical grade human OX40L:Ig to test in clinical trials for patients suffering from prostate cancer. With that goal in mind we made a GMP compliant cell line that produces large quantities of the protein within the first year of funding. Future work will include testing this protein in monkey primates for toxicology studies, which are typically mandated by the FDA prior to approval for phase I studies to be conducted in cancer patients.

BODY: The second year of funding was spent on understanding the importance of Fc-receptor binding on the anti-tumor efficacy of the OX40L:Ig fusion protein. In previous work funded by the DOD we characterized the optimal sequences that gave us the most potent biologic activity for the human OX40L:Ig fusion protein. This protein was subsequently tested in non-human primate studies and the potent biologic activity observed allowed for confidence to move forward with cell line production.

During this funding cycle, Fall 2011, we licensed the OX40L:Ig fusion program to an industry sponsor for testing in cancer patients. The license deal is a collaborative project between the two groups and is especially advantageous in terms of increased support for the program as far as taking this platform through phase I, II, and III clinical trials. The sponsor's protein chemistry group has taken several antibodies and Ig fusion proteins to the clinic and has vast experience communicating with the FDA to gain approval for clinical testing. Upon evaluating the OX40L:Ig sequence their group noticed that we had left the Fc gamma-binding domain of the Ig portion intact in the construct we produced. The industry sponsor mutated those sequences so that they no longer bound the Fc gamma receptor and the OX40L:Ig fusion protein with the mutated sequence had a reduced capacity to enhance Fc-mediated NK killing in an in vitro assay. While they felt that mutating the OX40L:Ig sequence would lead to a "safer" profile for FDA screening purposes, it was not clear whether mutating this sequence would decrease the anti-tumor efficacy of the OX40L:Ig protein. Therefore both groups agreed that further testing of the OX40L:Ig fusion protein was warranted in tumor models. To this end we have produced the identical murine version of the OX40L:Ig fusion protein and we tested its anti-tumor efficacy in mice that are devoid in Fc-receptor binding during the second year of funding.

In order to determine whether Fc-receptor engagement is important for the activity of an OX40 agonist we obtained mice that lack the activating Fc-receptors, termed Fcer1g ko mice. The activating Fc-receptors are defined by having the common a γ chain that has an ITAM domain involved with transducing proinflammatory signals upon engagement. There are three Fc-receptors in mice that express the common γ chain and they are FcγRI, FcγRIII, and FcγRIV. All three are of these receptors are absent in the Fcer1g ko mice. WT and Fcer1g ko mice were inoculated with the MCA205 tumors, which is a tumor that responds well to OX40 agonist therapy. We administered the murine OX40L:Ig fusion protein and an agonist OX40 Ab, OX86, on days 3 and 7 after tumor inoculation and followed the mice for tumor growth. As shown in Figure 1A the number of mice rejecting tumors was significantly less in the Fcer1g ko mice compared to WT mice for both the anti-OX40 Ab and the OX40L:Ig fusion protein. This finding was also evident regarding increased survival of the OX40 agonist treated WT mice compared to the ko mice as shown in the Kaplan-Meir plots in Fig 1B. In an initial attempt to understand the mechanism regarding why Fc-receptors are important for the

OX40 agonist efficacy, we phenotyped T cell subsets known to be involved with tumor immunotherapy at several time points after OX40 agonist stimulation. In particular, we found that there was a significant increase in the percentage of peripheral blood T regulatory cells (Treg) seven days post-OX40 agonist administration in the Fcer1 ko mice compared to WT mice (Figure 2A-C). We also found that the proliferation marker, Ki-67, was significantly increased in Tregs isolated from the Fcer1 ko mice following OX40 agonist stimulation compared to treatment of WT mice (Figure 2D). The result is of interest because it is known that an increase in Tregs typically correlates with a negative outcome regarding tumor immunotherapy. We will be testing whether the OX40L:Ig fusion protein can also an increase in Treg numbers and proliferation in tumor-bearing hosts in the coming months.

There is also an Fc-receptor ko mice that deletes only the inhibitory receptor (FcγRIIB) and therefore we testede whether the anti-OX40 therapy was abrogated in this ko mice strain. As shown in Figure 3 we found no statistical difference in survival when we compared the OX40 agonist in tumor-bearing WT vs FcIIB ko mice. We also tested whether there was an increase in Treg proliferation in the FcIIB ko mice. As opposed to what was observed in the Fcer1g ko mice there was no increase in Treg percentage or proliferation (data not shown). Hence, the data supports the hypothesis that OX40 agonists bind the activating Fc-receptor(s), which in turn impart biologic function to help the immune system reject tumors.

The data presented in this report was shared with our corporate partners and based on the results they produced two human versions of the OX40L:Ig fusion protein that both interact with human Fc receptors. The two forms that were chosen have different Fc tails but since the OX40L:Ig protein is hexameric complex with six Fc tails they interact more efficiently than an Ab with Fc receptors. The two forms of the OX40L:Ig fusion proteins, one of which was the original IgG1 construct, were tested in monkeys for potency as detected by increased CD4 and CD8 T cell proliferation. The company found that the second construct was significantly more potent than the original IgG1 construct in regards to both CD4 and CD8 T cell proliferation. Based on this data our partner has nominated the new OX40L:Ig construct as a drug candidate and will be performing a monkey toxicology study the will commence in the first quarter of 2014. If the toxicology study is successful they hope to file an IND with the OX40L:Ig fusion protein to the FDA and treat patients soon thereafter. Hence in the final year of this funded application we will focus on the optimal dose regimen that will allow for immune-mediated rejection of tumors using the OX40L:Ig fusion protein. Once the optimal dosing scheme is obtained we will test combinations with other immune enhancing agents in order to optimize the immunotherapeutic potential of OX40 agonists in tumor-bearing hosts

KEY RESEARCH ACCOMPLISHMENTS:

- The Fc-receptor binding portion of the OX40L:Ig fusion protein confers biologic activity that is important for its anti-tumor efficacy in vivo.
- The anti-tumor activity conferred by the Fc-receptor binding portion of the OX40L:Ig fusion protein is mediated through the activating Fc-receptors (FcγRI, FcγRIII, and/or FcγRIV).
- Based on the data Fc-receptor binding data to human OX40L:Ig fusion proteins were produced with two different Fc tails and one of them showed superior activity when tested in non-human primates.

REPORTABLE OUTCOMES:

The experiments planned within this proposal are to develop an OX40L:Ig fusion protein that can be injected into cancer patients. Hence, most of the experiments somewhat confirm what is already known in the literature and thus may be difficult to publish. However the data we generated regarding the OX40L:Ig fusion protein needing to bind the Fc-gamma receptor for biologic activity is a novel outcome, which could be a published in the future.

CONCLUSIONS:

In summary, we have shown that the OX40L:Ig fusion protein and an OX40 agonist Ab need to interact with Fc-receptors in order to in part their full anti-tumor activity. These results were obtained when OX40 agonists were tested in mice devoid of activating Fc-receptors as well as the inhibitory Fc-receptor. We found that the anti-tumor activity was associated with the activating Fc-receptor(s) and not the inhibitory receptor. We also found that the decreased activity in the Fc-recptor ko mice was associated with a significant increase in peripheral blood Treg numbers and proliferation. We now have the financial backing from an industry sponsor that will help take this protein into clinical trials. They have found that an OX40L with a particular Fc domain had the most potent in vivo activity and they will be performing an extensive monkey toxicology study with this agent in the coming months.

Figures:

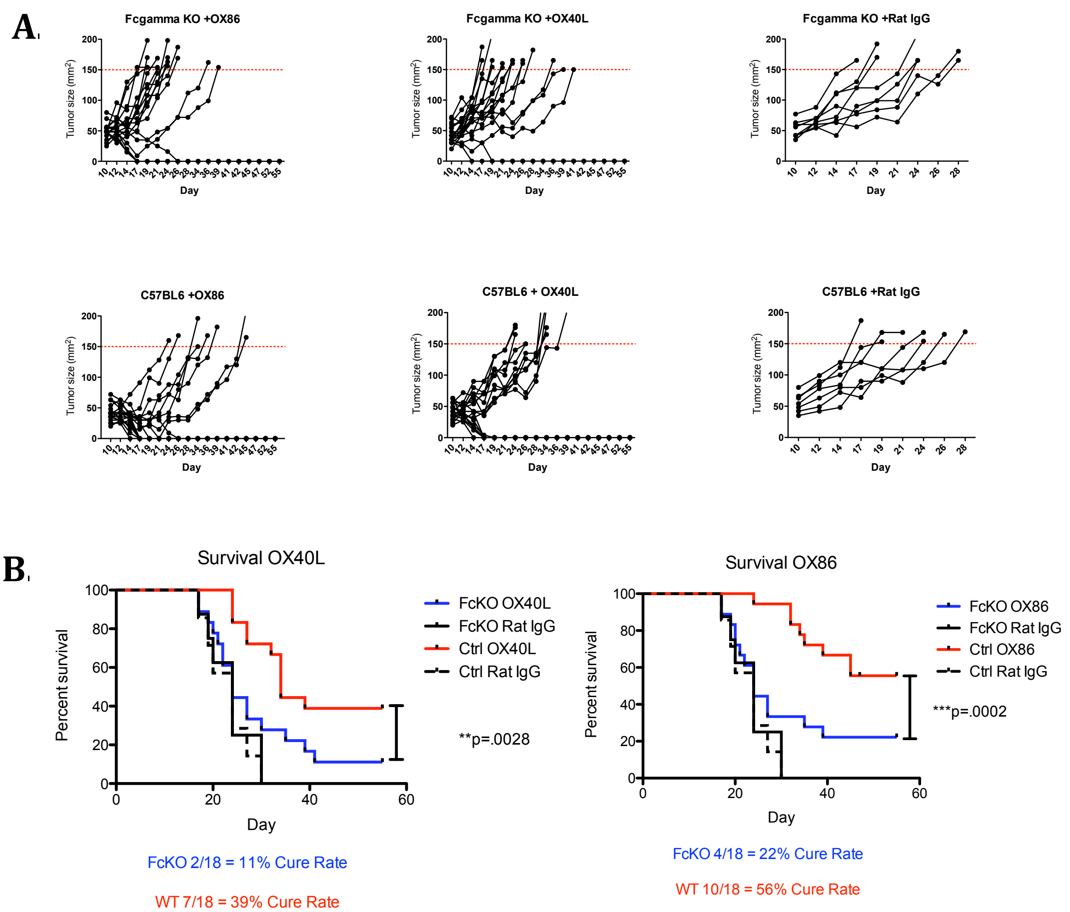
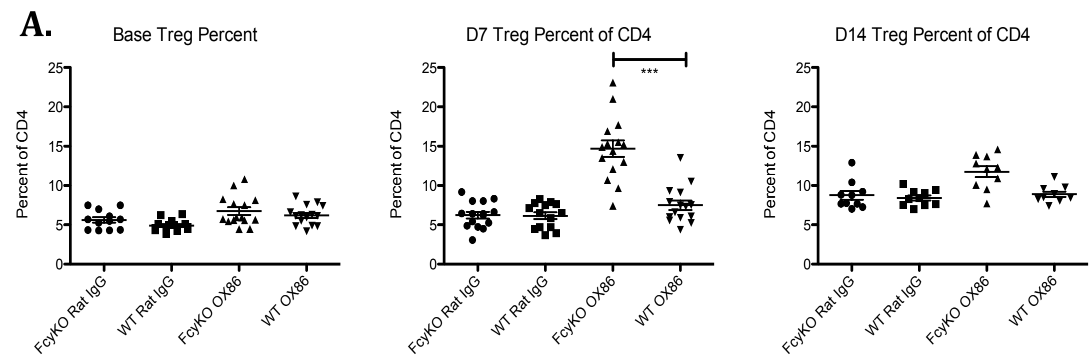


Figure 1: OX40 agonist therapy is impaired in mice lacking Fc-receptors. WT or Fcer1ko mice were injected with MCA205 tumors s.c. and treated with two injections of an OX40 agonist 3 and 7 days later. Mice received two doses at 250 ug of the OX40L:Ig fusion protein, anti-OX40 (OX86), or rat Ig. The mice were followed for tumor growth and were sacrificed when the tumor reached 150 mm2 or ulcerated. The graphs in panel A represents tumor growth curves while graphs in panel B show survival plots from the same experiment.



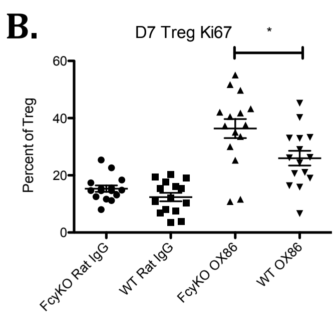


Figure 2: Increased OX40-induced Treg proliferation in Fc-receptor ko mice. Mice were injected with MCA205 tumors and treated with anti-OX40 or rat Ig as in Figure 1. Peripheral blood lymphocytes were isolated and the CD4/FoxP3 positive T cells (Treg) were assessed by flow cytometry for percent Tregs over time in panel A and percent of Tregs expressing Ki-67 on day 7 in panel B. Each dot on the graphs represent an individual mouse and 10 mice/group was used for the experiment.

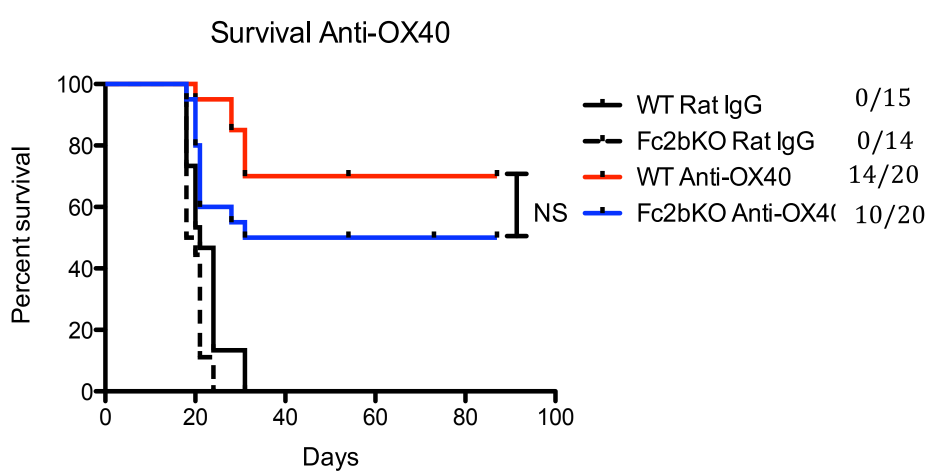


Figure 3: OX40 agonist therapy is not impaired in mice lacking the FcIIB-receptor. WT or FcRIIB ko mice were injected with MCA205 tumors s.c. and treated with two injections of OX40 agonist 3 and 7 days later. Mice received two doses at 250 ug of anti-OX40 (OX86) or rat Ig. The mice were followed for tumor growth and were sacrificed when the tumor reached 150 mm2 or ulcerated. The numbers shown in the figure indicate the number of tumor-free mice at day 80 after tumor inoculation/the total number of mice used to perform the experiment.